Supplementary Information

Truncating mutations of *MAGEL2* cause Prader-Willi phenotypes and autism

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Supplementary Note 1

Clinical Summaries

PATIENT 1

Patient 1, a Caucasian male, was seen at the age of 13 years in the genetics clinic for evaluation of pervasive developmental disorder and borderline intellectual functioning.

PREGNANCY AND BIRTH HISTORY: He was born at full term to a 40 yo G4P3A1 mother by vaginal, forceps-assisted delivery. Apgar scores were 9 and 9. Birth weight was 7 pounds and 12 ounces. He was doing well postnatally and discharged home on day of life 2 or 3. He had a **unilateral undescended testicle**, for which he underwent orchidopexy. He had **mild pes equinovarus** on the left.

MEDICAL HISTORY: The mother reports that she noted during early infancy that the child was **hypotonic**. He had a poor suck and **feeding difficulties** (breast and bottle) during months 1-3. He was thin as an infant. However, no specific measures had to be taken (no tube feeding). He struggled with **constipation** as an infant.

At 3 months, the boy was diagnosed with hypotonia by a nurse practitioner. He received physical and occupational therapy since infancy. At around 1.5 years, the child was seen by a geneticist, and as part of that evaluation **Prader-Willi methylation testing** was sent, the results of which were **negative**.

The boy has displayed **delayed attainment of developmental milestones**: sat at 9 months, walked at 2.5 years, spoke his first word at 3 years, talked in sentences at 4 years. Early childhood intervention was started at 6-9 months. He has not had any regressions.

At the age of 5 years, patient 1 was diagnosed with **Pervasive developmental disorder, NOS**. He has restricted interests, and displays many repetitive behaviors, has mannerisms, does a lot of stimming, rocking, hand wringing, etc. He is very social, trusting, has no fears. No skin picking.

Regarding his growth, he has been at approximately the 25th percentile for height, and between the 75th to 90th percentile for weight. He likes to eat, and his BMI is currently 22.3, which puts him in the overweight, but non-obese category. He does not have any significant food-seeking behaviors.

PHYSICAL EXAMINATION:

Weight %ile: 79th percentile based on weight-for-age.

Height %ile: 48th percentile based on stature-for-age.

OFC: 70th-75th percentile based on OFC-for-age.

General: Awake, alert, no acute distress. Some cognitive impairment. Lots of stimming and repetitive behaviors throughout the examination.

HEENT: Normocephalic. The face appears dysmorphic with a large mouth and some coarseness. Eyes are normal in shape, slanting and appearance. Pupils are equal, round and reactive to light. Ears are normal in shape, placement and rotation. Palate intact. Normal

uvula.

Neck: supple.

Chest: Normal in shape and configuration. Clear to auscultation bilaterally.

CVS: Regular in rate and rhythm. No murmurs, rubs or gallops appreciated on auscultation.

Abdomen: Soft, non-tender. No hepato-splenomegaly.

GU: Normal male genitalia. Bilateral testes down in the scrotum. Tanner stage IV.

Back: Scoliosis.

Extremities: No deformations. All digits intact. Warm and well perfused, with brisk capillary refill. Tapering fingers bilaterally. Total hand length 17.6 cm (left) and 17.7 cm (right). Palm length 10.8 cm (left) and 11 cm (right). These measurements are all within normal limits. Feet are 23 cm (left) and 23.2 cm (right), which are between the 3rd and 25th percentiles for age. He displays some hyperextensibility and is double-jointed in multiple joints. On the Beighton scale, his score is 2 of 9 (for extension >90 degrees of both 5th fingers).

Neurological Exam: Cranial nerves appear grossly intact. There is normal muscular strength and bulk. Deep tendon reflexes are normal and symmetric, slightly brisk (3/4). Sensation appears intact. No cerebellar signs, no ataxia, no dysdiadochokinesis, no dysmetria. No abnormal movements noted on examination.

PATIENT 2

Patient 2, a Hispanic male, was first seen by the genetic inpatient consultation service at the age of 2 years and 8 months for an evaluation of obesity, history of bilateral cryptorchidism, and developmental delay.

PREGNANCY and BIRTH HISTORY: The pregnancy is reported as uncomplicated. Ultrasounds done at 4, 6 and 9 months were normal. Mom was 37 years old at pregnancy. Amniocentesis was not preformed at mom's request. Quadruple test was interpreted as normal. Baby was born at full term with spontaneous vaginal delivery. At birth, he was diagnosed with **congenital hip dysplasia**. He was discharged with his mother on day of life 3. Five days later, he was readmitted for UV treatment of neonatal jaundice and discharged after 3 days. He had **bilateral undescended testicles**.

MEDICAL HISTORY: Congenital hip dysplasia treated with a casting for 3 months. He was breastfed for the first 3 months. Mom stopped breastfeeding because of a urinary tract infection that required her to take oral antibiotics. In the months to follow, the baby was thought to have **poor suck** and was not gaining weight. He was admitted to the hospital and evaluated for **failure to thrive** (FTT). His feeding improved using a special nipple and he was discharged. His mother reports that as part of the FTT evaluation, he was found to have **poor muscle tone**. Chromosome analysis was performed and was reported as normal. At 12 to 18 months of age, he demonstrated a moderate degree of **hyperphagia and accelerated weight gain**.

SURGICAL HISTORY: Left orchiopexy at age 2 years 4 months. Right orchiopexy and right inguinal hernia repair at 2 years 8 months.

DEVELOPMENT: Sitting independently at 12 months, crawling at age of 16 months, first steps at age 2 years and 3 months. First words were at 18 months. At the time of evaluation (2 years, 8 months), he does not communicate verbally.

PHYSICAL EXAMINATION:

OFC 50th-75th percentile

Weight 22.5 kg at the age of 2 years and 8 months (far above the 97th percentile; 50th percentile age 6.5y)

Height: just below 95th percentile

Weight for stature: far above 95th percentile

BMI: 23 (far above 95th percentile for age)

HEENT: Normocephalic. Facies round and full with bitemporal narrowing and almond-shaped palpebral fissures.

NECK: Supple without adenopathy.

LUNGS: Clear to auscultation.

CARDIAC: Regular rate and rhythm with normal S1 and S2. No murmur audible.

CHEST: Bilateral gynecomastia.

ABDOMEN: Obese, soft. Bowel sounds heard. No hepatosplenomegaly or masses.

GU: Hypoplastic appearing male genitalia, small phallus. Testis surgically descended.

EXTREMITIES: Small appearing hands and feet. No polydactyly.

NEUROLOGIC: Awake. Patellar reflexes +2 bilaterally. Babinski negative.

Patient 2 was re-evaluated at the age of 8 years and 2 months:

This is a 8-year 2-month-old male with **obesity, history of bilateral cryptorchidism, developmental delay, cognitive impairment, behavioral concerns, and severe obstructive sleep apnea**.

Since his first evaluation at 2 years of age, he had Prader-Willi-Syndrome methylation testing, which was normal, as well as chromosome microarray analysis (normal). He has developed severe obstructive sleep apnea, mild central apnea, and an abnormal EEG showing a single burst of generalized spike and slow wave activity which is considered a "potentially epileptogenic" finding.

He rarely socializes with the other children and likes to be alone. He has repetitive behaviors, rocking, rubbing, and he meets DSM-IV criteria for autism spectrum disorder. He is unable to read or write. He enjoys music. He does not play with toys.

PHYSICAL EXAMINATION:

Height on the 90th percentile, weight 71.8 kg (far greater than 97th percentile), BMI 39.47 (far greater than 97th percentile), OFC on the 95th percentile.

PATIENT 3

Patient 3, a Caucasian male, was seen at the age of 5 years in the genetics clinic for evaluation of **developmental delay, cryptorchidism**, and distal digital congenital **contractures**.

PREGNANCY AND BIRTH HISTORY: He was the product of a natural conception. The pregnancy is reported as uncomplicated, and exposures to medications, cigarettes, drugs, alcohol are denied. He was born with mild distal contractures, **micropenis and bilateral undescended testicles**, small umbilical hernia.

MEDICAL HISTORY: This child was noted to have significant **feeding difficulties** postnatally, and placement of a nasogastric feeding tube was required. Given the lack of improvement, a percutaneous gastrostomy tube was placed. While he later started eating by mouth, the G-tube remains in place at the age of 5 years for administration of medications. His lowest weight was around the 25th percentile, but during early childhood, he manifested much increased appetite and his weight rapidly increased to >95th percentile. He is short, was diagnosed with **growth hormone deficiency**, and started on growth hormone supplementation at age 2 years. He had strabismus, for which he required patching. He has severe global developmental delay. A formal diagnosis of **autism spectrum disorder** was made. Beyond autism, his behaviors are notable for temper tantrums, spitting and pinching. He has been on medications to control aggressive behavior since age 5 years.

For his joint contractures, he has been receiving physical and occupational therapy, and splints. He has bilateral dislocated radial heads. He has mild elbow contractures, limiting his range of motion.

At the age of 11 months, Prader-Willi-Syndrome was entertained as a diagnosis, but methylation testing was negative. He also had normal chromosome microarrays, Fragile X testing, and sequencing for Rubinstein-Taybi Syndrome, as well as Pitt-Hopkins Syndrome.

PHYSICAL EXAMINATION:

OFC 50th-75th percentile

Weight 75th percentile

Height: 20th percentile

Body mass index: 17.8 (93rd percentile for age)

GENERAL: non-verbal with some receptive language.

HEENT: Normocephalic. Thin, blond hair. Eyes are deep set, with a subtle upslant. Nasal bridge is high, nose has a prominent, curved tip. Columella is prominent. Patient has a wide mouth, large tongue. Ears are normally set and shaped.

NECK: Supple without adenopathy.

LUNGS: Clear to auscultation.

CARDIAC: Regular rate and rhythm with normal S1 and S2. No murmur audible.

ABDOMEN: Wide umbilicus, s/p umbilical hernia repair. G-tube in place. No hepatosplenomegaly or masses.

GU: Normal appearing, Tanner I. Testes surgically descended.

EXTREMITIES: Elbow contractures limiting the range of motion slightly. Hands with mild camptodactyly, with slight ulnar deviation bilaterally. Fingers tapered.

NEUROLOGIC: Low muscle tone. Toddler gait. Deep tendon reflexes symmetric and equal.

PATIENT 4

This is a 19 y.o. Hispanic man, who was seen in the genetics clinic for evaluation of **intellectual disability, seizures, and obstructive sleep apnea**.

PREGNANCY AND BIRTH HISTORY: He was born at full term to a 18 yo G1P1 mother by vaginal delivery. The pregnancy is reported as uncomplicated and exposures (drugs, alcohol, nicotine) are denied. Prenatal ultrasounds are reported as normal. The delivery was vaginal and at full term, but there was failure to progress, leading to an attempt of forceps delivery, and then vacuum extraction. Birth weight was 7 lbs. The neonate was limp at the time of delivery. Apgar scores are not remembered. He needed suction and O2 for a while. There was a single seizure in the immediate postnatal period.

MEDICAL HISTORY: The patient had **neonatal hypotonia** and **poor suck,** so he required feedings by nasogastric tube for about 2 weeks. He was discharged home at 2 weeks. The mother reports that, at home, he was still too weak to breastfeed, so he was bottlefed. There was no continued need for tube feeding. An MRI of his brain at 1 month of age showed punctate cerebellar hemorrhage, but no other abnormalities.

At around 1 year of age, **developmental delay** and **growth retardation** became apparent. He sat at 8 months, crawled at 1 year, walked at 2 years, never talked. At the age of 3 or 4 years, he was underweight, and a high calorie diet was recommended. He was late to go into puberty, which started around 15 years old. However, now, at the age of 19 years, he is Tanner stage V.

Mother reports that the patient got chubby as he got older. He loves to eat. She reports that he basically always wants to eat. He does not show any signs of fullness, and when he has finished a plate, he will indicate that he wants more. However, his intellectual and executive functioning does not allow him to go and get food by himself.

Patient 4 has **severe intellectual disability**. He is not reported as aggressive. He has multiple autistic traits. He is non-verbal, does not make eye contact. He does not initiate play. He has multiple self-stimulating behaviors, including rocking, hand flapping, clapping, etc. Mother and aunt report that he does show some restricted areas of interest. He meets DSM-IV criteria for **autism spectrum disorder**.

The patient has **moderate to severe obstructive sleep apnea**. He does not tolerate a CPAP machine. Mom does report episodes of severe snoring, breath holding, grunting, and waking up. He will have a repeat sleep study, which is already scheduled.

PHYSICAL EXAMINATION:

Weight: 48.1 kg, which is <1st percentile based on weight-for-age (50th percentile for a 13.5 year-old).

Height: 148.5 cm, which is <1st percentile based on stature-for-age (50th percentile for a 12 year-old).

OFC: 52.6 cm, which is <1st percentile based on OFC-for-age (-2.6 SD)

BMI = 22, which is 36th percentile for age. Given the individual's growth deficiency, an age-based BMI percentile might not be appropriate. Based on the individual's height, which is on the 50th percentile for a 12 year-old boy, it might be more appropriate to use the percentiles for a 12 year-old. Based on that, the BMI would be on the 89th percentile.

Additional anthropomorphic measurements:

Palm length: 9.8 cm (< 3rd percentile, 50th percentile for a 12 year-old)

Total hand length: 15.5 cm (<< 2nd percentile, 50th percentile for a 10 year-old).

Foot length: 20.2 cm (>> 2nd percentile, 50th percentile for an 8 year-old).

General: Awake, alert, no acute distress. Severe intellectual disability.

HEENT: Normocephalic. He has mild dysmorphic features, mostly for a large and broad nose. Eyes are normal in shape, slanting and appearance. Pupils are equal, round and reactive to light. Long eyelashes. Ears are normal in shape, placement and rotation. Mouth normal. Palate intact. Dentition normal. Frontal incisors are 7.8 mm and 7.5 mm broad (within normal range).

Neck: supple.

Chest: Normal in shape and configuration. Clear to auscultation bilaterally.

CVS: Regular in rate and rhythm. No murmurs, rubs or gallops appreciated on auscultation.

Abdomen: Soft, non-tender. No hepatosplenomegaly.

GU: Normal male genitalia. Bilateral testes are descended. He is Tanner stage V.

Back: Intact.

Extremities: All digits intact. However, he has significant contractures/camptodactyly of both hands. Warm and well perfused, with brisk capillary refill.

Skin: He has a patch of excessive hair growth on his left upper thigh.

Neurological Exam: Cranial nerves appear grossly intact. The neurological exam is limited due to lack of cooperation. However, strength is good, and there are no focal neurological abnormalities evident.

Patient 4 did not consent to have photographs published in a medical journal.

Supplementary Figure 1
Patient photographs

Informed consent was obtained to publish these photographs.

Subject 1, infant

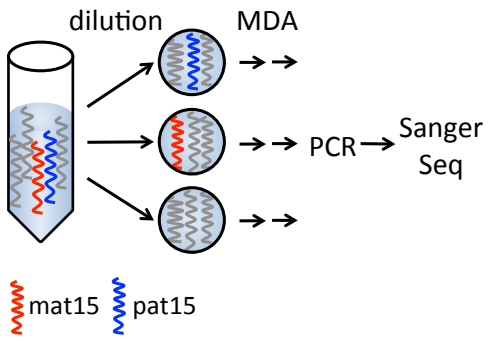
Subject 1, 13 years

Subject 2, 8 years

Subject 3, 5 years

Supplementary Figure 2

Phasing of the *MAGEL2* mutation in subject 1



paternal variant	T	G	G	?	A	
maternal variant	A	C	A	?	T	
Well	-12,785	-506	-422	deletion locus	+8,032	**inferred fragment length**
F11	+	+	pat	del	+	> 20 kb
F16	+	+	pat	del	+	> 20 kb
P23	+	+	pat	del	+	> 8 kb
I2	+	+	pat	del	+	> 12 kb
J12	+	+	pat	del	+	> 20 kb
P4	+	+	mat	T	+	> 20 kb
C16	-	+	pat	del	+	> 12 kb
L11	+	mat	mat	T	-	> 12 kb
I24	mat	mat	mat	T	-	> 12 kb
K10	mat	mat	mat	T	-	> 12 kb
D19	-	+	mat	T	-	> 20 kb
O4	-	pat	pat	del	mat	> 8 kb
O23	-	pat	pat	del	pat	> 8 kb
P8	-	pat	pat	del	pat	> 8 kb

Red color indicates maternal chromosome 15 (figure) and maternal allele (table), blue color indicates paternal chromosome 15 and allele, respectively. Wells containing fragments of chromosome 15 flanking the *de novo* deletion were identified by qPCR amplification of heterozygous SNPs; the qPCR fragments were then sequenced to determine the heredity of the fragment. A plus sign indicates a particular well was positive by qPCR for a particular SNP but sequencing either failed or was not performed; "mat" indicates the presence of a maternally-inherited SNP; "pat indicates the presence of a paternally-inherited SNP. The T allele at the deletion locus (no deletion) is always co-aliquoted with maternally-inherited SNPs, and with the exception of well O4, the deletion allele is always co-aliquoted with paternally-inherited SNPs, suggesting that the *de novo* deletion occurred on the paternally-inherited allele. Well O4 likely contains both maternal and paternal fragments of chromosome 15.

Supplementary Table 1 Comparison of Prader-Willi syndrome associated clinical features

Comparison between 4 cases reported herein and small deletion cases within the PWS locus reported in the literature

Prader-Willi syndrome diagnostic criteria based on Holmes et al. (1993)

Reference	**This manuscript**	**This manuscript**	**This manuscript**	**This manuscript**	**This manuscript**	**Sahoo et al, 2008**	**de Smith et al, 2009**	**Duker et al, 2010**	**Kanber et al, 2009**	**Kanber et al, 2009**	**Kanber et al, 2009**
Patient	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Summary**	**Patient 1**	**Patient 1**	**Patient 1**	**Patient 1**	**Patient 2**	**Patient 3**
Mutation type	1 bp deletion	1 bp deletion	2 bp deletion	nonsense		Deletion (174 kb)	Deletion (187 kb)	Deletion (236 kb)	Unbalanced translocation, causing monosomy 15pter-15q11.2	Deletion (6.5 Mb)	Deletion (5.0 Mb)
***MAGEL2* affected?**	yes	yes	yes	yes		no	no	no	yes	no	no
***SNORD116* affected?**	no	no	no	no		yes	yes	yes	no	yes	yes
Clinical Criterion											
Neonatal hypotonia, poor suck	1	1	0	1	3/4	1	1	1	1	1	1
Feeding problems in infancy, with need for special feeding technique	0	1	1	1	3/4	1	0	1	0	1	1
Excessive weight gain before 6 years	1	1	1	0	3/4	1	1	1	0	1	0
Hyperphagia	0	1	0	1	2/4	1	1	1	0	1	1
Developmental delay, Intellectual disability	1	1	1	1	4/4	1	1	1	1	1	1
PWS characteristic facial features	0	1	0	0	1/4	1	1	0	0	0	1
Hypogonadism	1	1	1	0	3/4	1	1	1	0	1	1
Deletion 15q11-13	0	0	0	0	0	0	0	0	0	0	0
Minor											
Infantile lethargy, weak cry	0.5	0	0.5	0.5	3/4	0	0	0.5	0	0	0.5
Short stature	0	0	0.5	0.5	2/4	0	0	0	0	0.5	0
Small hands	0	0	0	0.5	1/4	0.5	0.5	0	NR	0.5	0.5
Narrow hands	0	0	0	0.5	1/4	NR	NR	0	NR	NR	NR
Eye abnormalities (esotropia, myopia)	0	0.5	0.5	0.5	3/4	NR	NR	0.5	NR	NR	0.5
Hypopigmentation	0	0	0	0	0/4	0	0	0	0	0	NR
Thick saliva	0	0	0	0	0/4	NR	NR	0.5	NR	NR	0.5
Characteristic behavior (temper tantrums, violent outbursts, oppositional behavior, etc)	0	0	0.5	0.5	2/4	0.5	0.5	0	NR	NR	NR
Sleep disturbance or sleep apnea	0	0.5	0	0.5	2/4	0.5	0	NR	0	NR	0.5
Speech articulation defects	0	0.5	0.5	0.5	3/4	0.5	NR	0.5	NR	0.5	0.5
Skin picking		0	0.5	0.5	2/4	0.5	0.5	0	NR	NR	0.5
Supportive											
High pain threshold	yes				1/4			yes	yes		yes
Decreased vomiting				yes	1/4						
Temperature instability											
Scoliosis or kyphosis	yes				1/4				yes		yes
Early adrenarche									yes		
Osteoporosis											
Unusual skill with jigsaw puzzles											
Normal neuromuscular studies											
Total Score	**4.5**	**8.5**	**7**	**8.5**		**9.5**	**7.5**	**8**	**2**	**7.5**	**9.5**
Meets PWS criteria	**no**	**yes**	**no**	**no**		**yes**	**no**	**yes**	**no**	**no**	**yes**

Supplementary Table 2
List of all primer sequences – please refer to supplementary methods for experimental details

Primer Name	Primer sequence (5' to 3')	Purpose
Val1_Fw	GGGGGTAGCTGGATTTGCACGGCTTTT	Validation of c.1652delT
Val1_Re	TCCCCTGACTTGGCAGACCACGCAG	Validation of c.1652delT
pSNP-12785qPCRseqFw	GGTATGGGGAAGGATGGAGT	Phasing of c.1652delT
pSNP-12785qPCRseqRe	GCTGCTTTATCCAACCTCCT	Phasing of c.1652delT
mSNP-506-422qPCRseqFw	GGAGGTCCTGCGCTCTTTAG	Phasing of c.1652delT
mSNP-506-422qPCRseqRe	CAGGCCCAGGCATCGGGTCC	Phasing of c.1652delT
mSNP+8032qPCRseqFw	TGTAGCCTTCCAGATTGGCT	Phasing of c.1652delT
mSNP+8032qPCRseqRe	GGGGAACCAAGGGGTGTAT	Phasing of c.1652delT
magel11f	TGGAGTCATCAATGATTTAGCGGAG	Amplification of c.1652delT region
magel11r	AGGCACCAGCCGTTACCTGG	Amplification of c.1652delT region
LR_magel2_for	GAGAATTCCACCATCGCCACTAACC	Long range PCR for phase determination following *SmaI* digestion
LR_magel2_rev	CAGTCCCTGCAACTTCCCACTTTCT	Long range PCR for phase determination following *SmaI* digestion
Nested_PCR1_for	AGGCCCCGCCGCTGA	Nested PCR 1 for phase determination
Nested_PCR1_rev	GTGGGCACCTCCGCTTG	Nested PCR 1 for phase determination
Nested_PCR2_for	CCCGGTGGTTCCGATGAC	Nested PCR 2 for phase determination
Nested_PCR2_rev	CTGGGTCATCATGGCTGCT	Nested PCR 2 for phase determination